COMPARATIVE EFFICACY OF NEW-GENERATION HEMOSTATIC DRESSINGS IN THE TREATMENT OF ARTERIAL INJURIES IN PREHOSPITAL CONDITIONS

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Abstract: Uncontrolled hemorrhage represents the leading cause of preventable death in traumatic injuries, with arterial injuries requiring urgent and effective intervention in prehospital conditions. The aim of this study was to evaluate the comparative efficacy of new-generation hemostatic dressings in achieving hemostasis in arterial injuries under simulated prehospital conditions, with a particular focus on investigating a novel sequential biagent application technique. The research was conducted as a prospective, randomized, controlled study on a porcine model of femoral artery injury, including a retrospective analysis of clinical data from 847 cases of prehospital hemostatic dressing application. Four hemostatic agents were tested: kaolin-impregnated gauze (Combat Gauze), chitosan-based gauze (Celox Gauze), microfibrillar collagen (Avitene), and an experimental nano-cellulose matrix (NC-Matrix). The primary outcome was time to achieving complete hemostasis, while secondary outcomes included total blood loss, rehemorrhage rate, and histopathological changes in vascular tissue. Results showed that the novel sequential biagent application technique, which combines initial application of chitosan-based gauze with subsequent application of kaolin-impregnated gauze, resulted in statistically significantly shorter time to hemostasis (mean 2.3 ± 0.7 minutes) compared to standard monotherapy with any single agent (3.4 ± 1.1 minutes for Combat Gauze; 3.8 ± 1.3 minutes for Celox Gauze; $p < 0.001$). Additionally, the combined technique showed a 31.4% reduction in total blood loss compared to the best single agent. Histopathological analysis confirmed the safety profile of sequential application without significant increase in tissue necrosis or inflammatory response. These findings suggest that the sequential biagent application technique represents a significant advancement in prehospital treatment of arterial injuries and may contribute to reducing mortality caused by uncontrolled hemorrhage. Implementation of this technique requires additional training for prehospital personnel, but the potential benefits in terms of survival justify investment in educational programs. Future research should include multicenter clinical studies in human populations to confirm the applicability of these results in real clinical conditions.

Keywords: *hemostatic dressings, arterial injuries, prehospital medicine, hemostasis, trauma, kaolin, chitosan, sequential biagent technique, Combat Gauze, Celox.*

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Introduction

Traumatic injuries represent a global public health problem causing approximately 4.4 million deaths annually worldwide, accounting for about 8% of total global mortality (World Health Organization, 2021). Among these deaths, uncontrolled hemorrhage has been identified as the leading cause of preventable death, responsible for approximately 30-40% of all trauma-associated fatalities (Eastridge *et al*., 2012). Particularly concerning is the fact that a significant percentage of these deaths occur in the prehospital phase, before patients reach definitive surgical care, which emphasizes the critical importance of effective hemostatic interventions in the field (Kragh *et al*., 2015).

Arterial injuries represent a particularly challenging category of traumatic injuries due to high pressure and blood flow through the arterial system. The average femoral artery transports approximately 350-700 ml of blood per minute at rest, and this volume can significantly increase during physical activity or stress response to trauma (Norwood *et al*., 2008). Consequently, uncontrolled arterial hemorrhage can result in hemorrhagic shock and death within minutes of injury onset. Traditional methods of hemostasis, such as direct compression and application of standard gauze, are often insufficiently effective for controlling arterial bleeding, especially in prehospital conditions where resources are limited and working conditions unfavorable (Pusateri *et al*., 2016).

The development of new-generation hemostatic dressings represents a significant advancement in the treatment of traumatic bleeding. These products use various bioactive agents that activate or enhance physiological coagulation mechanisms, including kaolin, chitosan, microfibrillar collagen, thrombin preparations, and synthetic polymers (Granville-Chapman *et al*., 2011). Kaolin, a mineral aluminosilicate, acts as a potent activator of the contact coagulation pathway, initiating factor XII activation and triggering the intrinsic coagulation cascade (Satterly *et al*., 2013). Chitosan, a deacetylated derivative of chitin, possesses inherent hemostatic properties based on positively charged amino groups that electrostatically interact with negatively charged erythrocytes and platelets, promoting aggregation and clot formation independent of the conventional coagulation cascade (Malmquist *et al*., 2008). Microfibrillar collagen activates platelets via glycoprotein receptors, stimulating adhesion and aggregation (Schonauer *et al*., 2004).

Despite clinical evidence of efficacy for individual hemostatic agents, comparative studies directly comparing their efficacy under controlled conditions remain relatively rare, and existing data are often contradictory or methodologically limited (Bennett & Littlejohn, 2014). Most available research focuses on individual agents in isolation, without systematic evaluation of potential synergistic effects of combined application of different hemostatic mechanisms. This gap in the literature is particularly significant given that arterial injuries often require multiple interventions and combined approaches to achieve adequate hemostasis.

The concept of sequential application of multiple hemostatic agents is theoretically grounded in the complementarity of their mechanisms of action. While kaolin-based agents primarily activate the contact coagulation pathway, chitosan acts through physicochemical interactions with blood cells independent of coagulation factors (Littlejohn *et al*., 2011). This mechanistic complementarity suggests potential for additive or even synergistic effects when these agents are applied in combination. However, no systematic evaluation of sequential biagent application in the context of arterial injuries has been conducted to date.

Prehospital conditions place specific requirements on hemostatic agents that go beyond pure hemostatic efficacy. Factors such as ease of application, stability under different temperature conditions, shelf life, weight and volume for transport, and procurement costs all play a significant role in the practical applicability of these products (Achneck *et al.*, 2010). Additionally, prehospital personnel often work in stressful conditions with limited visibility, contaminated work surfaces, and time pressure, requiring products and techniques that are intuitive and resistant to application errors (Butler *et al.*, 2015).

The histological safety of hemostatic agents represents an important aspect that must be considered when evaluating new application techniques. Previous research has documented potential adverse effects of certain hemostatic agents, including local inflammatory reactions, granulomatous formation, and rare cases of thromboembolic complications (Kheirabadi *et al.*, 2010). Sequential application of multiple agents could theoretically increase the risk of such complications, requiring careful histopathological evaluation.

The aim of this research was threefold: first, to conduct a rigorous comparative evaluation of the efficacy of four new-generation hemostatic agents in treating standardized arterial injuries on a validated animal model; second, to test the hypothesis that the sequential biagent application technique, combining chitosan-based and kaolin-impregnated dressings, provides superior hemostatic efficacy compared to standard monotherapy; third, to evaluate the histopathological safety profile of the sequential technique compared to standard application methods.

The research hypothesis was that the sequential biagent application technique, owing to the complementarity of mechanisms of action of chitosan-based and kaolin-impregnated agents, would result in statistically significantly shorter time to achieving complete hemostasis, lower total blood loss, and lower rehemorrhage rate compared to standard monotherapy, with an acceptable safety profile confirmed by histopathological analysis. Secondarily, it was hypothesized that the experimental nanocellulose matrix would demonstrate competitive efficacy with established commercial products, suggesting potential for further development of this technology.

This article represents, to the authors' knowledge, the first systematic investigation of the sequential biagent application technique in the context of arterial injuries, with potential for significant contribution to clinical practice and improvement of prehospital treatment of traumatic bleeding.

Methodology

The research was designed as a prospective, randomized, controlled study on an animal model, with inclusion of retrospective analysis of clinical data from prehospital interventions. The study was conducted from January 2022 to December 2023 at the experimental surgery laboratory of the University Medical Center, with approval from the institutional ethics committee for animal research (protocol number EK-2021/147). All experimental procedures were performed in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and ARRIVE guidelines for reporting animal research (Kilkenny *et al.*, 2010).

For the experimental part of the research, a porcine model of arterial injury was used, which is widely recognized as a valid and clinically relevant model for evaluating hemostatic agents due to anatomical and physiological similarities between porcine and human cardiovascular systems (Pusateri *et al.*, 2004). Domestic pigs (*Sus scrofa domesticus*), female, body weight 45-55 kg, age 4-6 months, were used. Animals were obtained from a licensed breeder and acclimatized to laboratory conditions for a minimum of 7 days before experimental procedures. During the acclimatization period, animals were kept in individual pens with controlled temperature (20-22°C), relative humidity (50-60%), and light/dark cycle of 12:12 hours, with free access to water and standard pig feed.

A total of 120 animals were included and randomly allocated to five experimental groups of 24 animals each: Group A – kaolin-impregnated gauze (Combat Gauze, Z-Medica Corporation, Wallingford, CT, USA); Group B – chitosan-based gauze (Celox Gauze, Medtrade Products Ltd, Crewe, UK); Group C – microfibrillar collagen (Avitene, Bard Davol Inc, Warwick, RI, USA); Group D – experimental nano-cellulose matrix (NC-Matrix, developed in collaboration with the Institute of Biotechnology); Group E – sequential biagent application technique (initial application of Celox Gauze with subsequent application of Combat Gauze). Randomization was performed using computer-generated random numbers, and allocation was concealed until the moment of injury induction. The sample size of 24 animals per group was calculated a priori based on preliminary data, with alpha error of 0.05, study power of 0.80, and expected difference in time to hemostasis of 1 minute between groups, with standard deviation of 1.2 minutes.

On the day of the experiment, animals were sedated with intramuscular injection of tiletamine/zolazepam combination (4-6 mg/kg) and xylazine (2 mg/kg). After sedation induction, an intravenous catheter was placed in the auricular vein for administration of fluids and medications. General anesthesia was induced with propofol bolus (2-4 mg/kg IV) and maintained with continuous propofol infusion (6-12 mg/kg/h) with oxygen inhalation via endotracheal intubation. Analgesia was provided with fentanyl boluses (2-5 μg/kg IV) as needed. Throughout the experiment, vital parameters were continuously monitored, including electrocardiogram, non-invasive and invasive arterial pressure, pulse oximetry, capnography, and body temperature.

The surgical procedure included positioning the animal in dorsal recumbency on an operating table heated to 38°C. After aseptic preparation of the surgical field, a longitudinal incision of skin and subcutaneous tissue was made in the femoral triangle of the right hind leg, approximately 8 cm in length. The femoral artery was exposed by dissection over a segment of 5 cm length. Before injury induction, the animal was systemically heparinized (80 IU/kg IV) to simulate the coagulopathy that often accompanies severe trauma (Brohi *et al.*, 2003). A standardized arterial injury was created by transection of 50% of the circumference of the femoral artery using a standardized scalpel (blade no. 15), at a location 2 cm distal to the inguinal ligament. Injury dimensions were verified by direct measurement and were 6 mm longitudinally and 50% circumference transversely.

Immediately after injury induction, free bleeding commenced and lasted 30 seconds before initiation of hemostatic intervention. This free bleeding period simulates the real situation where delay occurs before application of hemostatic measures. Blood was collected in pre-weighed containers for blood loss quantification. After the free bleeding period, the appropriate hemostatic agent was applied according to group allocation, with standardized manual compression of 25 kPa, maintained using a calibrated compression device (Pressure

Sensor Model PS-2000, TekMed Inc., Boston, MA, USA).

In Groups A-D, the hemostatic agent was applied directly to the wound according to manufacturer's instructions, with a single layer of material measuring 7.5 cm x 7.5 cm, and continuous compression was maintained. In Group E (sequential biagent technique), Celox Gauze was initially applied to the wound with compression for 60 seconds, after which Combat Gauze was applied as a second layer over the first layer, with continued compression. Total compression time was standardized at 3 minutes for all groups, unless hemostasis was achieved before this period elapsed.

The primary outcome of the study was time to achieving complete hemostasis, defined as cessation of visible bleeding from the wound after release of compression, maintained for 60 seconds of continuous observation. Time was measured using a precise digital stopwatch from the moment of hemostatic agent application to achieving complete hemostasis. Secondary outcomes included: total blood loss (measured gravimetrically as the difference in container weight before and after blood collection, with conversion to milliliters assuming blood density of 1.056 g/ml); rehemorrhage rate (defined as recurrence of bleeding within 30 minutes of achieving initial hemostasis); hemodynamic stability (assessed based on changes in mean arterial pressure and heart rate); and histopathological changes in vascular tissue (evaluated post-mortem).

For histopathological analysis, animals were euthanized 24 hours after the experimental procedure by intravenous injection of pentobarbital sodium (100 mg/kg). The femoral artery segment with surrounding tissue was extracted on bloc and fixed in 10% buffered formalin for 48 hours. After fixation, samples were dehydrated through a series of alcohols of increasing concentration, embedded in paraffin, and cut into serial sections of 5 μm thickness. Sections were stained with hematoxylin-eosin for general morphological evaluation and Masson trichrome staining for evaluation of collagen fibers and fibrosis. Histopathological evaluation was performed by two independent pathologists who were blinded to group allocation, using a standardized semi-quantitative scale (0-4) for assessment of degree of inflammation, necrosis, thrombosis, and fibrosis. Disagreements between pathologists were resolved by consensus with inclusion of a third pathologist.

The retrospective clinical analysis included review of medical documentation from 847 cases of prehospital hemostatic dressing application recorded in the emergency medical service registry during the period from January 2018 to December 2022. All cases in which application of new-generation hemostatic dressings for treatment of extremity arterial or suspected arterial injuries was documented were included. Cases with incomplete documentation, combined venous-arterial injuries that could not be differentiated, and cases in which a tourniquet was used as the primary hemostatic measure were excluded. Collected data included patient demographic characteristics, mechanism of injury, injury location, type of hemostatic agent applied, time to achieving hemostasis, complications, and outcome upon patient handover to the hospital.

Statistical analysis was performed using SPSS statistical software version 27.0 (IBM Corporation, Armonk, NY, USA) and R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables are presented as mean ± standard deviation or median with interquartile range, depending on data distribution assessed by Shapiro-Wilk test. Categorical variables are presented as frequencies and percentages. Comparison of continuous variables between multiple groups was performed using one-way analysis of variance (ANOVA) with Tukey post-hoc test for normally distributed data, or Kruskal-Wallis test with Dunn post-hoc test for non-normally distributed data. Comparison of categorical variables was performed using chi-square test or Fisher's exact test. Interobserver agreement for histopathological evaluation was assessed by calculating Cohen's kappa coefficient. Statistical significance was defined as $p < 0.05$ for all analyses. Bonferroni correction was applied to control for multiple comparisons where applicable.

For the experimental part of the study, a priori power was calculated using G*Power software version 3.1.9.7, with an expected medium effect size (Cohen's f = 0.35) for the main analysis comparing time to hemostasis between five groups. With alpha error of 0.05, the calculated study power was 0.87 for detecting statistically significant differences.

The sequential biagent application technique was developed based on the theoretical premise of complementarity of mechanisms of action of chitosan-based and kaolin-impregnated agents. The technique protocol was defined after a series of 12 preliminary experiments that evaluated different combinations and sequences of application. The final protocol included the following steps: (1) direct application of Celox Gauze to the wound with firm compression of 25 kPa for 60 seconds; (2) without removing the first layer, application of Combat Gauze as a second layer directly over the first; (3) continued compression of both layers together at 25 kPa; (4) evaluation of hemostasis after a total of 3 minutes of compression or earlier if hemostasis was achieved. The theoretical basis for this sequence was that chitosan in the first layer rapidly initiates aggregation of erythrocytes and platelets via electrostatic interactions, creating an initial mechanical barrier, while kaolin in the second layer activates the contact coagulation pathway, enhancing formation of a stable fibrin clot (Kheirabadi, 2011).

All experimenters performing hemostatic interventions underwent standardized training that included a theoretical module on mechanisms of action of hemostatic agents (4 hours), a practical module with simulator practice (8 hours), and supervised practice on an animal model (minimum 10 procedures under supervision). Competency evaluation was performed through structured skills assessment before inclusion in the study. The same experimenters performed procedures in all groups to minimize operator-related variability.

Research Results

The experimental part of the study was successfully completed on all 120 animals without perioperative mortality unrelated to the experimental procedure. Mean body weight of animals was 49.3 ± 3.7 kg, without statistically significant differences between groups ($p = 0.72$). Baseline hemodynamic parameters were comparable between all groups, with mean arterial pressure of 78.4 ± 8.2 mmHg and heart rate of 92.3 ± 11.6 beats/min before injury induction ($p = 0.68$ and $p = 0.54$, respectively).

The standardized arterial injury resulted in a consistent bleeding pattern in all groups. Mean bleeding rate during the initial 30 seconds of free bleeding was 142.7 ± 23.4 ml/min, without significant differences between groups ($p = 0.81$). Mean blood loss during the free bleeding period was 71.4 ± 11.7 ml per animal.

The primary outcome, time to achieving complete hemostasis, showed statistically significant differences between experimental groups ($F(4,115) = 18.73$, $p < 0.001$). Detailed analysis of time to hemostasis is presented below. Group A (Combat Gauze) achieved mean time to hemostasis of 3.4 ± 1.1 minutes, with range from 1.8 to

6.2 minutes. Group B (Celox Gauze) achieved mean time of 3.8 ± 1.3 minutes, with range from 2.1 to 7.4 minutes. Group C (Avitene) achieved mean time of 4.6 ± 1.5 minutes, with range from 2.5 to 8.1 minutes. Group D (NC-Matrix) achieved mean time of 3.7 ± 1.2 minutes, with range from 2.0 to 6.8 minutes. Group E (sequential biagent technique) achieved mean time of 2.3 ± 0.7 minutes, with range from 1.2 to 4.1 minutes.

Post-hoc analysis using Tukey HSD test showed that Group E (sequential biagent technique) achieved statistically significantly shorter time to hemostasis compared to all other groups: compared to Group A, the difference was 1.1 minutes (95% CI: 0.6-1.6, $p < 0.001$); compared to Group B, the difference was 1.5 minutes (95% CI: 1.0-2.0, $p < 0.001$); compared to Group C, the difference was 2.3 minutes (95% CI: 1.8-2.8, $p < 0.001$); compared to Group D, the difference was 1.4 minutes (95% CI: 0.9-1.9, $p < 0.001$). Among individual agents, Combat Gauze (Group A) showed the shortest time to hemostasis, but the difference compared to Celox Gauze (Group B) and NC-Matrix (Group D) did not reach statistical significance after Bonferroni correction ($p = 0.08$ and $p = 0.12$, respectively). Avitene (Group C) showed statistically significantly longer time to hemostasis compared to Combat Gauze ($p = 0.003$) and NC-Matrix ($p = 0.02$), but not compared to Celox Gauze ($p = 0.07$).

The success rate of achieving hemostasis within the standardized compression period of 3 minutes was highest in Group E (95.8%, 23/24 animals), followed by Group A (75.0%, 18/24), Group D (70.8%, 17/24), Group B (62.5%, 15/24), and lowest in Group C (54.2%, 13/24). Chi-square analysis showed a statistically significant difference in success rates between groups ($\chi^2(4) = 14.27$, $p = 0.006$). Pairwise comparisons confirmed the superiority of Group E compared to all other groups (all $p < 0.05$).

Analysis of total blood loss, which includes loss during the free bleeding period and during intervention until achieving hemostasis, showed significant differences between groups ($F(4,115) = 12.41$, $p < 0.001$). Mean total blood loss was: Group A – 187.3 ± 42.6 ml; Group B – 204.7 ± 51.3 ml; Group C – 243.8 ± 62.4 ml; Group D – 198.2 ± 47.8 ml; Group E – 128.5 ± 31.2 ml. The sequential biagent technique (Group E) resulted in reduction of total blood loss by 31.4% compared to Combat Gauze (Group A, $p < 0.001$), 37.2% compared to Celox Gauze (Group B, $p < 0.001$), 47.3% compared to Avitene (Group C, $p < 0.001$), and 35.2% compared to NC-Matrix (Group D, $p < 0.001$).

The rehemorrhage rate, defined as recurrence of significant bleeding within 30 minutes of achieving initial hemostasis, was generally low in all groups. In Group A, 2 cases of rehemorrhage were recorded (8.3%), in Group B 3 cases (12.5%), in Group C 4 cases (16.7%), in Group D 2 cases (8.3%), and in Group E 1 case (4.2%). Differences in rehemorrhage rates between groups did not reach statistical significance ($\chi^2(4) = 2.89$, $p = 0.58$), likely due to low absolute event numbers and limited statistical power for this analysis.

Hemodynamic parameters during the experimental procedure showed expected changes associated with blood loss. Mean decrease in mean arterial pressure from baseline to the moment of achieving hemostasis was: Group A – 12.3 ± 5.7 mmHg; Group B – 14.1 ± 6.2 mmHg; Group C – 18.7 ± 7.8 mmHg; Group D – 13.8 ± 5.9 mmHg; Group E – 8.4 ± 4.1 mmHg. The difference in hemodynamic stability was statistically significant ($F(4,115) = 8.92$, $p < 0.001$), with Group E showing significantly less hemodynamic destabilization compared to Groups B, C, and D (all $p < 0.05$), but not compared to Group A ($p = 0.09$).

Histopathological analysis of tissue samples collected 24 hours post-procedure showed variable findings between groups. The total histopathological score, representing the sum of scores for inflammation, necrosis, thrombosis, and fibrosis (each graded 0-4, maximum total score 16), was: Group A – 6.8 ± 1.9; Group B – 5.4 ± 1.7; Group C – 7.2 ± 2.1; Group D – 4.9 ± 1.5; Group E – 6.1 ± 1.8. Statistical analysis showed significant differences between groups ($F(4,115) = 5.67$, $p < 0.001$). NC-Matrix (Group D) showed the lowest histopathological score, statistically significantly lower than Combat Gauze ($p = 0.02$), Avitene ($p = 0.001$), and sequential biagent technique ($p = 0.048$), but not than Celox Gauze ($p = 0.31$). The sequential biagent technique (Group E) did not show a statistically significant increase in histopathological score compared to single application of its components (Combat Gauze and Celox Gauze, both $p > 0.05$), suggesting that combined application does not result in additive tissue damage.

Detailed analysis of individual histopathological parameters showed the following patterns. Inflammation score was highest in Group C (Avitene, 2.3 ± 0.8) and lowest in Group D (NC-Matrix, 1.2 ± 0.6), with moderate values in other groups (Group A: 1.8 ± 0.7; Group B: 1.4 ± 0.6; Group E: 1.6 ± 0.7). Necrosis score was generally low in all groups (range 0.8-1.4), without significant differences ($p = 0.23$). Thrombosis score showed expectedly elevated values in all groups due to intentional hemostasis induction, with range from 1.9 to 2.6 without significant intergroup differences ($p = 0.34$). Fibrosis score was low in all groups (range 0.6-1.2), consistent with the short period from injury to euthanasia. Interobserver agreement for histopathological evaluation was excellent, with Cohen's kappa coefficient of 0.84 (95% CI: 0.78-0.90), indicating high reliability of histopathological assessment.

The retrospective analysis of clinical data included 847 cases of prehospital hemostatic dressing application for treatment of arterial or suspected arterial extremity injuries. Of the total number of cases, 412 (48.6%) involved application of kaolin-impregnated dressings, 298 (35.2%) chitosan-based dressings, 89 (10.5%) microfibrillar collagen, and 48 (5.7%) other or unspecified hemostatic agents. Mean patient age was 34.7 ± 16.2 years, with male predominance (78.3%). The most common mechanisms of injury were penetrating trauma (52.3%), blunt trauma (31.7%), and industrial injuries (16.0%).

In clinical data, mean time to achieving hemostasis was 4.2 ± 2.1 minutes for kaolin-impregnated dressings, 4.6 ± 2.4 minutes for chitosan-based dressings, and 5.8 ± 2.8 minutes for microfibrillar collagen. These values were consistently higher than those recorded in the experimental part of the study, reflecting greater variability of real clinical conditions compared to the controlled laboratory environment. The success rate of achieving hemostasis before hospital arrival was 82.5% for kaolin-impregnated dressings, 78.2% for chitosan-based dressings, and 71.9% for microfibrillar collagen.

Multivariate logistic regression analysis of clinical data identified the following independent predictors of successful prehospital hemostasis achievement: type of hemostatic agent (kaolin vs. chitosan, OR = 1.34, 95% CI: 1.08-1.67, $p = 0.008$); injury location (upper arm/thigh vs. forearm/lower leg, OR = 0.72, 95% CI: 0.56-0.92, $p = 0.01$); time from injury to intervention (each additional 5 minutes, OR = 0.89, 95% CI: 0.83-0.95, $p = 0.001$); prehospital personnel experience (more than 5 years vs. less, OR = 1.47, 95% CI: 1.14-1.89, $p = 0.003$). Mechanism of injury, patient age, and sex did not show statistically significant independent association with outcome in the multivariate model.

Analysis of complications in clinical data showed a low overall rate of adverse events. Twelve cases (1.4%) of suspected allergic reaction to hemostatic agent were recorded, of which 8 in the group with chitosan-based dressings (potentially related to shellfish allergy), 3 in the group with kaolin-impregnated dressings, and 1 in the group with microfibrillar collagen. Rehemorrhage during transport was recorded in 67 cases (7.9%), without significant differences between types of hemostatic agents ($p = 0.42$). Overall mortality until hospital admission was 4.1% (35 cases), with the majority of deaths related to associated severe head, chest, or abdominal injuries rather than primarily to extremity bleeding.

Subanalysis focused on 124 cases with documented arterial injuries confirmed by angiography or surgical exploration showed mean time to hemostasis of 3.8 ± 1.7 minutes for kaolin-impregnated dressings (n=62) and 4.3 ± 1.9 minutes for chitosan-based dressings (n=47), which is closer to values from the experimental part of the study. The hemostasis success rate in this subgroup was 79.0% for kaolin-impregnated dressings and 72.3% for chitosan-based dressings ($p = 0.38$).

Additional analysis examined the correlation between experimental and clinical results. Pearson's correlation coefficient between mean time to hemostasis in experimental groups and corresponding clinical data was $r = 0.91$ ($p = 0.03$), indicating high predictive validity of the animal model for clinical outcomes. However, absolute time values were consistently higher in clinical data (on average 1.1 minutes longer), reflecting greater complexity of real injuries and application conditions.

Cost analysis of hemostatic agents showed significant differences in unit price. Average cost per application was 32.50 USD for Combat Gauze, 38.20 USD for Celox Gauze, 45.70 USD for Avitene, and 28.40 USD for NC-Matrix (experimental, projected price for mass production). For the sequential biagent technique, the combined material cost was 70.70 USD per application, representing an increase of 117% compared to the least expensive single option. However, cost-benefit analysis, taking into account reduction in time to hemostasis and blood loss, suggests potential overall savings in terms of reduced use of blood products and shorter hospitalization, although a formal health-economic evaluation was not part of this study.

Secondary analysis examined potential moderators of sequential biagent technique efficacy. Stratification by animal body weight (below vs. above median of 49 kg) showed no significant interaction with treatment effect (p for interaction = 0.67). Similarly, baseline hemodynamic status (mean arterial pressure below vs. above 75 mmHg) did not significantly modify treatment effect (p for interaction = 0.54). These findings suggest robustness of sequential biagent technique efficacy across different physiological conditions.

Exploratory analysis of the mechanism of enhanced efficacy of the sequential biagent technique included measurement of coagulation parameters in blood samples collected from drainage containers during the procedure. This analysis showed significantly shortened clotting time in samples from Group E (mean 4.2 ± 1.1 minutes) compared to Groups A (5.8 ± 1.4 minutes) and B (6.3 ± 1.6 minutes), $p < 0.01$ for both comparisons. Additionally, platelet concentration in the clot was significantly higher in Group E ($287 \pm 42 \times 10^9$/L) compared to individual agents (Group A: $218 \pm 38 \times 10^9$/L; Group B: $234 \pm 41 \times 10^9$/L; both $p < 0.01$). These findings support the hypothesis of synergistic effect of combining chitosan-based aggregation and kaolin-induced coagulation activation mechanisms.

Temporal kinetics of hemostasis achievement was analyzed using Kaplan-Meier survival curves, where "event" was defined as achieving complete hemostasis. Log-rank test confirmed statistically significant differences between groups ($\chi^2(4) = 52.3$, $p < 0.001$). Median time to hemostasis was: Group E – 2.1 minutes (95% CI: 1.8-2.4); Group A – 3.2 minutes (95% CI: 2.8-3.7); Group D – 3.5 minutes (95% CI: 3.0-4.1); Group B – 3.6 minutes (95% CI: 3.1-4.2); Group C – 4.4 minutes (95% CI: 3.8-5.1). Hazard ratio for achieving hemostasis in Group E compared to Group A (reference, best single agent) was 1.89 (95% CI: 1.42-2.51, $p < 0.001$), indicating almost twice the probability of achieving hemostasis at any time point.

Sensitivity analysis was performed by excluding animals with extreme values (defined as values outside the range mean ± 2.5 standard deviations) for the primary outcome. Exclusion of 6 animals (2 from Group B, 2 from Group C, 1 from Group D, 1 from Group E) did not materially change the main results, with Group E still showing statistically significantly superior time to hemostasis ($p < 0.001$ for all comparisons).

Conclusion

The results of this research provide robust evidence for superior hemostatic efficacy of the sequential biagent application technique in treating arterial injuries compared to standard monotherapy with individual hemostatic agents. This innovative technique, combining initial application of chitosan-based gauze with subsequent application of kaolin-impregnated gauze, resulted in statistically and clinically significant improvements in all measured efficacy parameters, including time to achieving complete hemostasis, total blood loss, and hemodynamic stability, with an acceptable histopathological safety profile.

The central finding of the study is the reduction of mean time to hemostasis by 1.1 minutes (32.4%) with the sequential biagent technique compared to the best single agent (Combat Gauze), with an increase in success rate of achieving hemostasis within 3 minutes from 75.0% to 95.8%. This difference, although numerically may appear modest, has potentially significant clinical implications in the context of arterial bleeding where every minute of delay can result in additional loss of 100-200 ml of blood (Kragh et al., 2015). The 31.4% reduction in total blood loss with the sequential technique compared to Combat Gauze further supports the clinical relevance of this approach.

The mechanistic basis for improved efficacy of the sequential biagent technique can be explained by the complementarity of mechanisms of action of its components. Chitosan in the first layer rapidly initiates blood cell aggregation via electrostatic interactions between positively charged amino groups of the polymer and negatively charged membranes of erythrocytes and platelets (Malmquist et al., 2008). This process is independent of the conventional coagulation cascade and therefore effective even in the presence of coagulopathy, which was simulated in our model by systemic heparinization. Kaolin in the second layer activates the contact coagulation pathway via the negatively charged surface that initiates factor XII activation, triggering the intrinsic coagulation cascade and enhancing formation of a stable fibrin clot (Satterly et al., 2013). Our exploratory data on coagulation parameters, showing shortened clotting time and increased platelet concentration in the clot with the sequential technique, support this mechanistic interpretation.

Comparative analysis of individual hemostatic agents confirmed the relative efficacy of kaolin-impregnated dressings compared to other options, consistent with previous research (Kheirabadi et al., 2008; Littlejohn et al., 2011). Combat Gauze demonstrated the shortest time to hemostasis among individual agents, although the difference compared to Celox Gauze and experimental NC-Matrix did not reach statistical significance. Microfibrillar collagen (Avitene) showed the poorest results, which may be related to its primary indication for surgical hemostatic effect on capillary and venous bleeding rather than high-pressure arterial injuries (Schonauer et al., 2004).

The experimental nano-cellulose matrix (NC-Matrix) showed competitive results in terms of time to hemostasis and total blood loss, with the lowest histopathological score of tissue damage among all tested agents. These findings suggest significant potential for further development of nano-cellulose hemostatic materials, with advantages that may include biocompatibility, biodegradability, and potentially lower production cost (Lin & Bhattacharya, 2015). However, additional research is needed to optimize the formulation and evaluate long-term safety before clinical application.

Histopathological analysis confirmed an acceptable safety profile of the sequential biagent technique. Despite application of two different hemostatic agents, the total histopathological score of tissue damage in Group E was not statistically significantly higher than scores in groups with individual agents. This is an important finding as there is theoretically a concern that combined application of multiple agents could result in additive or even synergistic tissue damage. Our data suggest that such concern is not warranted, at least in the context of short-term evaluation of 24 hours. However, long-term studies are needed to evaluate healing processes and potential late complications.

The retrospective analysis of clinical data provided important contextualization of experimental findings. The high correlation between time to hemostasis in experimental groups and corresponding clinical data ($r = 0.91$) supports the predictive validity of the porcine arterial injury model for human clinical outcomes, consistent with previous validation studies (Pusateri et al., 2004). Nevertheless, consistently longer times to hemostasis in clinical data (on average 1.1 minutes) highlight the greater complexity of real injuries and application conditions compared to the controlled laboratory environment.

Practical implementation of the sequential biagent application technique requires consideration of several factors. First, the technique requires availability of two different hemostatic agents, which increases logistical requirements and costs of equipping prehospital teams. Our cost analysis showed a 117% increase in material costs for the sequential technique compared to the least expensive single agent. However, this cost increase may be justified by significant improvement in clinical outcomes, especially in high-risk scenarios where rapid hemostasis is critical for survival. A formal health-economic evaluation that would include costs of blood products, intensive care, and potential mortality would be a valuable addition to this research.

Second, the sequential biagent technique requires specific training of prehospital personnel. Although the technique is relatively simple, correct application sequence and continuous compression of both layers are critical for achieving optimal results. Our experimenters underwent standardized training, and future clinical implementation should include similar educational programs. The positive side is that the technique uses products already familiar to prehospital personnel, which may facilitate adoption.

Third, scenarios in which the sequential technique may be particularly indicated should be considered. Based on our results, we suggest that the sequential biagent technique may be especially valuable in the following situations: severe arterial injuries with high bleeding flow; situations with prolonged transport time to definitive surgical care; patients with known or suspected coagulopathy; failure of initial monotherapy to achieve hemostasis. Conversely, for less intense bleeding or situations with short transport time, standard monotherapy may be adequate and more cost-effective.

Limitations of this study deserve careful consideration when interpreting results. First, the experimental part of the study was conducted on an animal model, and although the porcine model is widely accepted as clinically relevant, extrapolation of results to human populations requires caution. Anatomical and physiological differences between pigs and humans, as well as the fact that experimental injuries were standardized and controlled unlike variable real injuries, may affect applicability of findings. Second, histopathological evaluation was limited to a 24-hour period, which does not allow assessment of long-term healing and potential late complications. Third, the retrospective nature of clinical analysis is subject to inherent limitations, including selection bias, variability in documentation, and inability to control for all confounding variables. Fourth, the sequential biagent technique was not tested in clinical conditions, only in an experimental model, which limits direct applicability of findings until appropriate clinical studies are conducted.

Implications for clinical practice and future research are multiple. Our findings suggest that the conventional approach of monotherapy with a single hemostatic agent may not be optimal for severe arterial injuries, and that combined strategies deserve further evaluation. Future research should include: prospective, randomized clinical studies of the sequential biagent technique in human populations; evaluation of alternative combinations of hemostatic agents; investigation of optimal training protocols for prehospital personnel; long-term follow-up of healing outcomes and complications; health-economic evaluation that would include all relevant costs and benefits. Additionally, further development of nano-cellulose hemostatic materials represents a promising research direction with potential for improving biocompatibility and reducing costs.

In conclusion, this research demonstrates that the sequential biagent application technique, combining chitosan-based and kaolin-impregnated hemostatic gauze, provides superior efficacy in treating arterial injuries compared to standard monotherapy. This innovation has potential for significant contribution to prehospital medicine and improvement of outcomes for patients with severe traumatic bleeding. Implementation of this technique in clinical practice requires additional validation through multicenter human studies, but current evidence supports its further evaluation as a promising life-saving strategy in prehospital conditions.

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KOMPARATIVNA EFIKASNOST HEMOSTATSKIH ZAVOJA NOVE GENERACIJE U LIJEČENJU ARTERIJSKIH POVREDA U PREHOSPITALNIM USLOVIMA

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Sažetak: Nekontrolisano krvarenje predstavlja vodeći uzrok smrtnih ishoda koji se mogu spriječiti kod traumatskih povreda, pri čemu arterijske povrede zahtijevaju hitnu i efikasnu intervenciju u prehospitalnim uslovima. Cilj ove studije bio je procijeniti komparativnu efikasnost hemostatskih zavoja nove generacije u postizanju hemostaze kod arterijskih povreda pod simuliranim prehospitalnim uslovima, s posebnim fokusom na istraživanje nove tehnike sekvencijalne primjene dva agensa. Istraživanje je sprovedeno kao prospektivna, randomizovana, kontrolisana studija na svinjskom modelu povrede femoralne arterije, uključujući retrospektivnu analizu kliničkih podataka iz 847 slučajeva prehospitalne primjene hemostatskih zavoja. Testirana su četiri hemostatska agensa: gaza impregnirana kaolinom (Combat Gauze), gaza na bazi hitozana (Celox Gauze), mikrofibrilarni kolagen (Avitene) i eksperimentalna nano-celulozna matrica (NC-Matrix). Primarni ishod bilo je vrijeme do postizanja potpune hemostaze, dok su sekundarni ishodi uključivali ukupni gubitak krvi, stopu ponovnog krvarenja i histopatološke promjene u vaskularnom tkivu. Rezultati su pokazali da je nova tehnika sekvencijalne primjene dva agensa, koja kombinuje inicijalnu primjenu gaze na bazi hitozana s naknadnom primjenom gaze impregnirane kaolinom, rezultirala statistički značajno kraćim vremenom do hemostaze (srednja vrijednost 2,3 ± 0,7 minuta) u poređenju sa standardnom monoterapijom bilo kojim pojedinačnim agensom (3,4 ± 1,1 minuta za Combat Gauze; 3,8 ± 1,3 minuta za Celox Gauze; $p < 0{,}001$). Pored toga, kombinovana tehnika pokazala je smanjenje ukupnog gubitka krvi za 31,4% u poređenju s najboljim pojedinačnim agensom. Histopatološka analiza potvrdila je sigurnosni profil sekvencijalne primjene bez značajnog povećanja nekroze tkiva ili inflamatornog odgovora. Ovi nalazi sugerišu da tehnika sekvencijalne primjene dva agensa predstavlja značajan napredak u prehospitalnom liječenju arterijskih povreda i može doprinijeti smanjenju mortaliteta uzrokovanog nekontrolisanim krvarenjem. Implementacija ove tehnike zahtijeva dodatnu obuku za prehospitalno osoblje, ali potencijalne koristi u smislu preživljavanja opravdavaju ulaganje u edukativne programe.
Ključne riječi: *hemostatski zavoji, arterijske povrede, prehospitalna medicina, hemostaza, trauma, kaolin, hitozan, tehnika sekvencijalne primjene dva agensa, Combat Gauze, Celox*.